Report for 2001CT621B: Development of Predictive Tools to Infer Inhibition of Biological Nitrogen Removal at POTWs vi Long Term Bench-Scale and Full-Scale Monitoring

- Conference Proceedings:
  - Hu, Z., K. Chandran, D. Grasso and B. F. Smets. 2002. "A comparative study of nitrification inhibition by heavy metals: the influence of metal exposure time on biological effect." in 8th Annual Industrial Waste Technical and Regulatory Conference, Atlanta City, NJ.
  - Chandran, K., Hu, Z, and B. F. Smets 2001. "Optimal Experimental Design for Estimating Ammonia and Nitrite Oxidation Biokinetics from Batch Respirograms". in 74th Annual Water Environment Federation Conference, 2001. Atlanta, GA.
  - Hu, Z, Chandran, K., B. F. Smets and Grasso, D 2001. "Evaluation of Nitrification Inhibition by Heavy Metals Nickel and Zinc". in 74th Annual Water Environment Federation Conference, 2001. Atlanta, GA.
- Articles in Refereed Scientific Journals:
  - Hu, Z., K. Chandran, D. Grasso, and B. F. Smets. 2002. Effect of nickel and cadmium speciation on nitrification inhibition. Environmental Science and Technology 36:3074-3078.
  - Hu, Z., K. Chandran, D. Grasso, and B. F. Smets. 2003. Impact of metal sorption and internalization on nitrification inhibition. Environmental Science and Technology 37:728-734.
  - Hu, Z., K. Chandran, D. Grasso, and B. F. Smets. 2003. Nitrification inhibition by ethylenediamine-based chelating agents. Environmental Engineering Science:Accepted for Publication.
  - Hu, Z., K. Chandran, D. Grasso, and B. F. Smets. 2003. Comparison of nitrification inhibition by metals in batch and continuous flow reactors. Water Research: In Preparation.
- Dissertations:
  - Hu, Z. 2002. "Nitrification inhibition by heavy metals and chelating agents." PhD. Dissertation, University of Connecticut, Storrs, CT.

Report Follows:

Title: Development of Predictive Tools to Infer Inhibition of Biological Nitrogen Removal at POTWs via Long Term Bench Scale and Full Scale Monitoring

Statement of Critical Regional of State Water Problem. The Long Island Sound , bordered by Long Island, Connecticut, and New York, is a vast recreational (e.g., boating) and economical (e.g., fish and shell fish harvesting, navigation) resource. Unfortunately, the Sound's ecosystem continues to be under tremendous stress, jeopardizing its current uses for the future. Although the reasons for the Sound's fragile ecosystem health are manifold, nutrient discharge in the Sound, especially nitrogen, is generally recognized as a key contributor to ecosystem deterioration [1]. **P**ublicly **o**wned **t**reatment **w**orks (POTWs) in Connecticut and New York present major point sources for nitrogen loads that can enter the local watershed and the Sound, resulting in hypoxic conditions in the Sound during late summers [2]. The Long Island Sound Comprehensive Conservation Management Plan requires that both Connecticut and New York reduce their nitrogen discharges to Long Island Sound by 58.5 % [1]. In an attempt to meet these new regulations, the Environmental Protection Agencies of Connecticut and New York are investing multi-million dollar amounts to upgrade wastewater treatment plants for **bi**ological **ni**trogen **r**emoval (BNR) with the aim of improving water quality in the Long Island Sound. Unfortunately, many POTWs that have already installed these BNR processes are experiencing intermittent and extended periods when there is a loss of either nitrification or denitrification [2]. Not only does this result in permit violations for the municipality, but also in high total nitrogen discharges to Long Island Sound.

In order to insure that the water quality in Long Island Sound is improved and to protect this major investment, it is vital to understand and identify the cause of failures of BNR processes and to identify the factors preventing individual treatment plants from establishing BNR. The goal of the proposed research is to provide this critical information to regulatory and policy setting agencies in the states of NY and CT as well as to the professionals responsible for the treatment of domestic and industrial waste streams in these two Long Island Sound bordering states.

Statement of Research Results or Benefit. The primary focus of this research is to identify the causes of poor biological nitrogen removal (BNR) in POTWs. This research will compliment an ongoing study on nitrification inhibition study to help identify determinants of N-removal failure at POTWs due to process control deficiencies and waste stream characteristics (*Inhibition of Biological Nitrogen Removal: Microbiology, Physical Chemistry and Process Engineering, by B. F. Smets, D. Grasso, and J. Semon-Brown, March 1999-March 2001*). Driven by an exhaustive literature review, and the results of the ongoing study, we have implicated nitrification, the biochemical oxidation of ammonium-N to nitrate-N, as the bottleneck in BNR. This study will permit the validation of developed assays to rapidly measure the kinetics of the nitrification reaction using mixed liquor from a full-scale treatment plant. Part of this validation will entail a comparison of measured kinetics with observed reactor performance of bench-scale and full-scale BNR reactors across several seasons. Validation of the developed nitrification kinetics assay will yield a rapid tool to measure kinetics of the appropriate BNR rate-limiting step in full-scale POTWs, hitherto not available.

In addition, this study will permit the validation of several analytical assays that are currently being developed to identify and quantify the presence of possible nitrification inhibitors in the complex reactor influents (raw domestic wastewaters) to full scale POTWs. These assays are geared towards measuring total heavy metal content, total cationic surfactant content, and total chelating agent content of a wastewater, expressed in terms of an appropriate normalizing equivalent. Such rapid tools will prove invaluable for rapid and intermittent screening of wastewaters to assess inhibitory character. Both kinetic and inhibitor quantification assays will be developed for transfer to wide use by POTWs across the state and region to rapidly identify and mitigate BNR inhibition.

Nature, Scope & Objective of Research. Under a LISS funded program, the University of Connecticut and the City of Stamford Water Pollution Control Authority (WPCA) are examining the contributions of process engineering (hydraulics, aeration, carbon and nutrient limitation), microbiology (kinetics and stoichiometry) and physical chemistry (nitrogen speciation, availability and matrix chemistry) to BNR limitation (*Inhibition of Biological Nitrogen Removal: Microbiology, Physical Chemistry and Process*

Engineering, by B. F. Smets, D. Grasso, and J. Semon-Brown, March 1999-March 2001). One of the primary objectives of that study is to develop predictive analytical and numerical tools that permit a rapid evaluation of BNR limitation. Consequently, with bench-scale nitrifying reactors at the University of Connecticut, fed with a defined inorganic medium, we are studying reactor performance and nitrification kinetics under undisturbed operation and in response to system perturbations, such as a transient load of toxic chemicals. We have developed and optimized batch respirometric assays to measure nitrification biokinetics from continuously operated bench-scale nitrifying reactors at the University of Connecticut [3, 4]. In addition, we are conducting shock load tests to the nitrifying reactors at UCONN to validate the biokinetic assays as rapid indicators of nitrification inhibition. Further, to enable examination of the complex dynamics of different microbial populations and substrates in actual wastewater treatment bioreactors and varying influent conditions and biocatalyst activity, we propose to monitor nitrogen removal in full-scale and bench-scale activated sludge bioreactor treating actual wastewater.
Nitrification, the first requisite step in BNR, is predominantly catalyzed by autotrophic bacteria and involves the oxidation of ammonium-nitrogen (NH_4^+-N) to nitrite-nitrogen (NO_2^--N) and nitrate-nitrogen (NO_3^--N) [5]. Denitrification, the second requisite step in BNR, is primarily mediated by heterotrophic bacteria, which biochemically reduce ionic nitrogen oxides such as NO_3^--N and NO_2^--N to gaseous nitric oxide (NO), nitrous oxide (N_2O) and nitrogen (N_2) and sometimes even to NH_4^+-N under extremely anaerobic conditions [6]. Due to their different substrate requirements, (*e.g.*, nitrification requires a minimum O_2 : NH_4^+-N ratio of around 4.3, minimum alkalinity (as $CaCO_3$) : NH_4^+-N ratio of around 8.6 and denitrification requires a minimum COD : NO_3^--N ratio of approximately 2.9), the presence and growth of nitrifying and denitrifying bacteria depends somewhat on wastewater composition. BNR can be promoted by engineering reactors to ensure robust nitrifying and denitrifying populations. For instance, a commonly used Modified LÜdzack Etinger (MLE) configuration for BNR consists of an anoxic reactor for denitrification fed with influent carbon and recirculating NO_3^--N from a downstream aerobic nitrification reactor [7]. The effect of process engineering on BNR efficacy can be evaluated and optimized based on certain key indicator stoichiometric ratios. As part of the ongoing project, we developed stoichiometric ratios that will permit a ready evaluation of different causes for the limitation of biological nitrogen removal. While some indicator ratios are either based on the wastewater composition, others arise from the design and operation of the biological nitrogen removal reactor (Tables 1-2). The effect of a range of the indicator stoichiometric ratios was evaluated by performing simulations of a Modified LÜdzak Ettinger process using BIOWIN 32™ (EnviroSim Associates, Flamborough, Canada) using default model parameters and wastewater composition.

Table 1 : Indicator stoichiometric ratios based on wastewater composition

Ratio (units)	Ratio describes
sCOD/NO_3^--N (mg sCOD/mg NO_3^--N)	Effect of influent soluble COD on denitrification
S_{alk}/NH_3-N (mg HCO_3^- alkalinity/mg NH_3-N oxidized to NO_3^--N)	Effect of influent alkalinity on nitrification
f_{na} (mg NH_3-N/mg tTKN)	Effect of nitrogen availability on nitrification

Table 2 : Indicator stoichiometric ratios based on process operation

Ratio (units)	Ratio describes

$\frac{O_2}{K_{O,A}+O_2}$ (mg O_2/mg O_2)	Effect of dissolved oxygen concentration in the aerobic zone on nitrification
$\theta_{C,}/\theta_{C,minimum}$ (Aerobic SRT provided/Minimum aerobic SRT required for nitrification)	Effect of reactor sizing on nitrification
f_{na} (mg NO_3^--N produced/mg NH_3-N oxidized)	Extent of nitrification under uninhibited or peak operation

It is commonly believed that nitrification is the bottleneck in BNR due to the slow growth kinetics and high susceptibility of nitrifying bacteria to numerous environmental disturbances [8]. Because of the inherent variability of wastewater composition and the dynamics of microbial communities, it is critical to develop tools to measure the activity of that fraction repeatedly, thereby necessitating an easy, rapid, yet accurate assay for routine monitoring. Further, in the same context, there is also a need for tools to rapidly screen a wastewater for potential inhibitors.

- Under the purview of the ongoing BNR limitation study, we have optimized a rapid respirometric nitrification assay to yield nitrification kinetic parameter estimates in a continuously operated bench-scale reactor containing an enriched nitrifying consortium [3, 4]. The developed assay relies on automated measurements of stoichiometric oxygen consumption in response to oxidation of a transient ammonia or nitrite spike. The resulting oxygen uptake profile is fit to Monod-based kinetic expressions to determine the respective kinetic constants q_{max} (maximum specific substrate consumption rate, 1/time) and K_S (half saturation coefficient, mg-N/L) [3, 4]. In the proposed study, we will validate application of the batch respirometric assay to measuring nitrification kinetics in a full-scale BNR reactor treating actual domestic and industrial wastewater, across a wide range of seasonal, wastewater composition and biocatalyst activity dynamics, during a prolonged monitoring campaign.
- In addition, we will develop rapid screening tools to measure wastewater composition and correlate bulk composition measures to nitrification inhibition. Initially, we will focus on metals (cadmium, zinc, nickel and copper, moderate to high toxicity to nitrifying microorganisms [5]), metal binding agents (EDTA, CDTA, NTA, high susceptibility of copper cofactor based ammonia monooxygenase due to copper non-availability [9, 10]), anionic surfactants (sodium lauryl sulfate, found in influent to the Stamford WPCA, Operations Management Inc., New Haven , CT, Personal Communication). The inhibitory character of several classes of compounds will be related to bulk measurable properties using artificial neural networks (ANN). (See Section 13 for details). During the next few months, prior to the start of the monitoring study, we will optimize and validate the bulk chemical characterization tool in an synthetic matrix, such as the cultivation medium for nitrifying bacteria [11]. Subsequently, a more stringent assessment will be conducted using the primary effluent to the treatment train at the Stamford WPCA, subject to the seasonal changes in wastewater composition.

Methods, procedures, and facilities

Respirometric Assay to Measure of Nitrification Kinetics . The kinetics of nitrification and nitrification inhibition will be measured using an extant respirometric assay developed in our laboratories [3, 4]. The developed assay measures the kinetics of nitrification exhibited by biomass derived from a continuously operated nitrifying enrichment culture [12]. The developed respirometric technique is based on stoichiometric consumption of oxygen in response to ammonia and nitrite oxidation [13]. In contrast to nitrification kinetic assays that depend on analytically involved and chemical-specific time-series measurement of nitrogen depletion (for *e.g.*,[14, 15]), the respirometric is rapid, reproducible and facile since oxygen measurements can be accurately performed and can be fully automated. An additional

feature of this method is that it allows distinction in activity of ammonia oxidizing and nitrite oxidizing microorganisms from a mixed nitrifying culture [12]. We are currently applying this technique to determine nitrification kinetics during undisturbed operation and during an imposed disturbance (Figs 1 and 2).

Reactor dynamics as measured by effluent concentrations (Fig 1) are strongly correlated with measured kinetic estimates (Fig. 2, SOUR is a measure of maximum NH_4^+-N and NO_2^--N oxidation capacity) : an SOUR increase in SOUR is accompanied by a decrease in effluent NH_4^+-N concentrations.

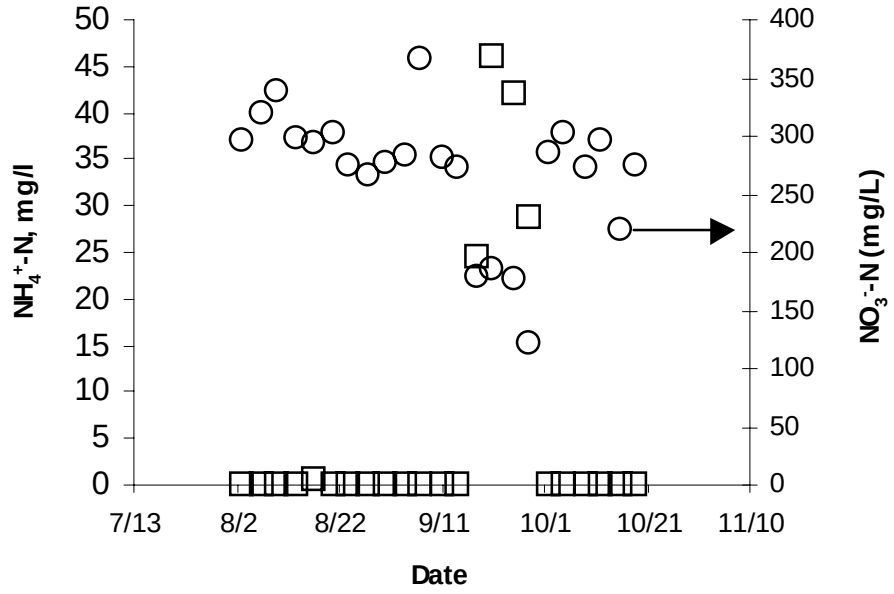

Figure 1 : Performance Profiles for Bench Scale Nitrifying Reactor. ( ) : NH_4^+-N concentrations and (O) : NO_3^--N concentrations.

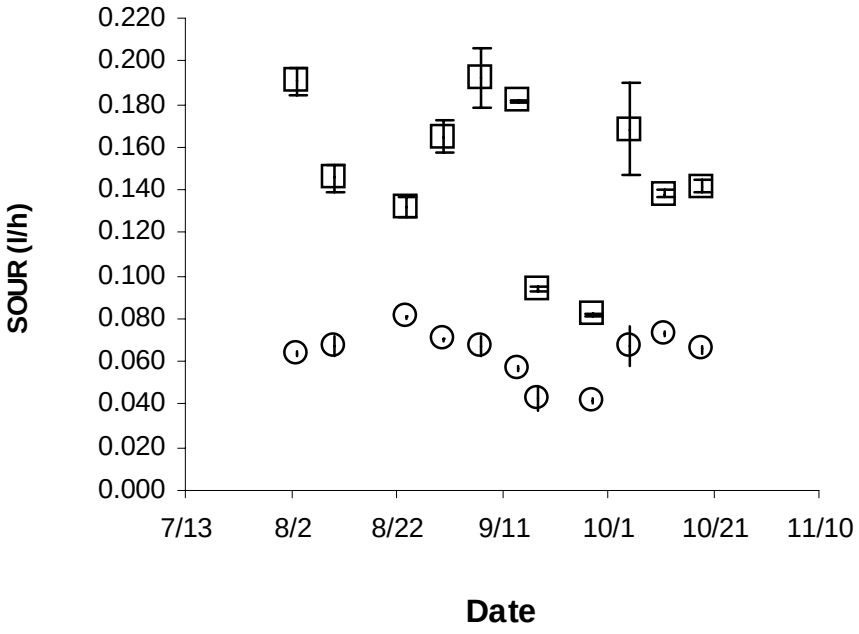

Figure 2 : Biokinetics Profiles for Bench Scale Nitrifying Reactor. ( ) : NH_4^+-N oxidation rate and (O) : specific NO_2^--N oxidation rate.

In addition, we have demonstrated in our laboratory that the proposed assay even permits determination of ammonia and nitrite oxidation kinetics estimates using actual activated sludge samples from a POTW, which contains a variety of microbial groups. We have optimized the developed respirometric assay to yield nitrification kinetic parameter estimates in a continuously operated reactor containing an enriched nitrifying consortium. In addition, in the proposed study, we will validate the method for activated sludge BNR reactors using an ASM2 model based simulation package (BIOWIN®, EnviroSim Asoociates Inc. Flamborough, Ontario, Canada) by evaluating the congruence of kinetic estimates against observed reactor performance.

Chemical analtyical MEASURES OF Nitrification Inhibitory character. We propose to develop quantitative structure-activity relationships (QSARs) that are based on measurable bulk properties of a wastewater sample, (*e.g.*, for metal binding capacity (chelating agents) surface active properties like critical micelle concentration (surfactants), anion binding capacity (metal ions)).
Different bulk characteristics will be developed to measure the presence of the above chemical classes in wastewater. The inhibitory action of heavy metals is due to interaction with enzymes containing thiol groups and formation of metal sulfide complexes [16]. The metal content of a wastewater matrix will be determined by titrating against excess sulfide (in an anoxic environment) and measuring remaining sulfide concentrations. The bulk measure of heavy metal content will be expressed in terms of a lumped metal-sulfide solubility product of the constituent metal-sulfide solubility product (Table 3).

Table 3 : Solubility Products of Metal Sulfides [17]

Metal	Log Solubility Product
Cadmium	-26.1
Zinc	-23.8 (□), -21.6 (□)
Nickel	-18.5 (□), -24 (□), -25.7 (□)
Copper	-47.6

Some metal chelating agents are postulated to function by rendering copper, (part of the ammonia monooxygenase cofactor) unavailable [10]. The presence of chelating agents in wastewater will be tested by titrating against copper or an alternate heavy metal (to be ascertained and optimized) and measuring residual ionic free concentration using ion selective electrodes. The bulk measure of chelator content will be expressed as an effective stability constant(s) for the metal-chelator complex, based on individual chelator-metal stability constants (Table 4).

Table 4 : Log Stability Constants for Chelator-Metal Complexes [18]

Chelator / **Metal**	**EDTA**	**CDTA**	**NTA**
Ba^{2+}	7.76	7.99	4.82
Ca^{2+}	10.7	12.5	6.41
Mg^{2+}	8.69	10.32	5.41
Zn^{2+}	16.26	18.67	10.45

The toxicity of cationic surfactants has been expressed in terms of their individual critical micelle concentrations (CMC) [19]. A similar approach will be followed for anionic surfactants. The bulk measure for the presence of surface active agents will be the ratio of the total surfactant concentration to the bulk CMC (via contact angle measurements).

The inhibitory character of several classes of compounds will be related to bulk measurable properties using artificial neural networks (ANN). The ANN will be trained using toxicity-property data obtained from batch biokinetic tests. The ANN QSAR model will be validated and verified by its ability to predict the toxicity due to mixture of different classes of compounds and an untested wastewater matrix.

We believe that our approach is superior to existing QSAR models, which are restricted to classification of organic compounds in terms of their homologous series, for *e.g.*, alcohols, halogenated aliphatics, alkanes, aromatics etc (for *e.g.*, [20]). While such a simplified classification is adequate for deterministic toxicity modeling of a well defined waste-stream, it is of limited application when dealing with a typical wastewater sample, wherein the constituent classes of compounds are unknown.

Operation of Bench-Scale BNR Reactors. To verify proposed measurement techniques, two bench scale bioreactors initiated during our current Long Island Research Foundation funded study, will be operated in parallel at the Stamford WPCA. The bioreactors have an operating volume of 40L, and a flow rate of 10 gallons per day. The reactors consist of one anoxic basin followed by three aerated basins, followed by an internal clarifier and were constructed at the Technical Services Center, University of Connecticut. These reactors will be operated under identical HRT, SRT, aeration and mixing regimes as the full-scale treatment WPCA (*i.e.* as a Modified LÜdzack Ettinger configuration). The bench-scale reactor operation was commenced in October 2000 on a side stream of the actual Stamford POTW wastewater influent. Once established, baseline nitrification and denitrification performance and kinetics in each of these reactors -operated in tandem- will be measured using chemical specific assays (Details can be found in Table 5) [21] and respirometric assays, respectively [3, 4].

Table 5 : Sampling Specifications for Bench-scale Reactors at Stamford WPCA

Name of Chemical or Method	Measurement Classification			Sample Location	Sample Preservation	Maximum Holding Time (Days)
	Type	Frequency (1/d)	Sample Equipment			
pH (Bench scale Reactor)	C	Not applicable	Not applicable	Reactor	NA	None, online
Chemical Oxygen Demand : Colorimetric	I, C	2/7 d	35 ml glass vial	Reactor	4^oC	1
NH_3-N Colorimetric	I, C	2/7 d	200 ml glass beaker	Reactor effluent	4^oC^{Ψ}	1
NO_2^--N Colorimetric	I, C	2/7 d	200 ml glass beaker	Reactor effluent	4^oC^{Ψ}	2
NO_3^--N Colorimetric	I, C	2/7 d	200 ml glass beaker	Reactor effluent	H_2SO_4, pH < 2	28
TKN Colorimetric	I,C	2/7 d	200 ml glass beaker	Reactor effluent	H_2SO_4, pH < 2	28
Biomass concentration (Mixed liquor suspended solids)	I, C	2/7 d	500 ml Erlenmeyer flask	Reactor	4^oC	1
Biomass concentration (Mixed liquor volatile suspended solids)	I,C	2/7 d	500 ml Erlenmeyer flask	Reactor	4^oC	1
Nitrification kinetics (Extant Respirometry)	I	As required	100 ml respirometric vessel	Reactor	4^oC	1
Dissolved oxygen (Bench scale Reactor)	C	Not applicable	Bench scale Reactor	Reactor	NA	None, Online

*: The tabulated sample volume is twice that required for routine duplicate analysis and is apportioned into two sample containers. The additional volume is collected to determine quality control measures such as accuracy (analysis of spiked samples), precision (duplicate analysis) and to account for potential sample loss while handling or analysis. (Also see section 1.7.5)

C : continuous measurement; I : intermittent measurement; Frequency of measurement applies only to continuous measurements

$^{\Psi}$: Storage at 4^oC. However, the biomass is removed from the sample via centrifugation at 3500 g for 10 minutes. Biomass removal arrests further biochemical oxidation of NH_4^+-N and NO_2^--N.

NA : Not applicable

** : Performed at the University of Connecticut
Frequency of measurement applies only to continuous measurements

Subsequently, one reactor will be subjected to different levels of a disturbance, (*e.g.* selected inhibitors will be added to the influent at several concentrations). Initially, we will choose anionic surfactants, such as sodium lauryl sulfate, since these have resulted in significant nitrification inhibition in POTWs in Stamford [2] and metal chelating agents (high susceptibility of metal cofactor based ammonia monooxygenase enzyme to compounds such as allylthiourea [9, 10]). In addition, as part of a parallel research investigation on heavy metal inhibition of nitrification, the bench-scale reactors will be dosed with nickel, copper, zinc and cadmium. During the imposed process disturbance, the BNR performance will be recorded. In addition, the proposed surrogate chemical measure of inhibitory character and biokinetics of nitrification and denitrification will be measured. The measured biokinetics will be input into the ASM2 model structure of BIOWIN®. The biokinetics and surrogate chemical measurement techniques will be confirmed by comparing measured nitrogen removal performance with that simulated by BIOWIN®. In the validation study, known nitrification inhibitors (e.g., metals, surfactants, metal chelators, and other compounds likely to occur in sewage waste streams) will selectively be added to one of the bench scale reactors. The ability to predict the effect of these inhibitors on nitrification kinetics via the bulk chemical measurements and developed QSARs will be assessed, while the kinetic assays will be verified to predict deterioriating reactor process performance prior to onset of reactor failure.

Evaluation of Full-Scale BNR performance. The treatment train at the Stamford Water Pollution Control Authority consists of two series of 2.46 million gallons volumetric capacity each. The total influent flow (average 20 million gallons per day) is equally split between the two trains. Each train consists of the equivalent of one anoxic and three aerobic reactors of 0.615 million gallons each. While bench scale testing is ongoing, the treatment train of the full scale Stamford POTW will be periodically sampled and analyzed (Details can be found in Table 6).

Table 6 : Sampling Specifications for Full-Scale Treatment Train at Stamford WPCA

Name of Chemical or Method	Measurement Classification			Sample Location	Sample Volume (ml)	Sample Preservation	Maximum Holding Time (Days)
	Type	Frequency (1/d)	Sample Equipment				
pH	C	8	1 L polypropylene bottle	1-5	10	Not applicable	Within 15 min of sampling
Chemical Oxygen Demand – Colorimetric	C	8	1 L polypropylene bottle	1-5	1000	4°C	7
NH_3-N Colorimetric	C,G	8,1	1 L polypropylene bottle	1-5	100	4°C$^{\Psi}$	1
NO_2^--N Colorimetric	C,G	8,1	1 L polypropylene bottle	1-5	100	4°C$^{\Psi}$	2
NO_3^--N Colorimetric	C,G	8,1	1 L polypropylene bottle	1-5	100	H_2SO_4, pH < 2	28
Soluble TKN Colorimetric	C,G	8,1	1 L polypropylene bottle	1-5	100	H_2SO_4, pH < 2	28
Biomass concentration Total suspended solids	C,G	8,1	1 L polypropylene bottle	1-5	1000	4°C	7
Nitrification Kinetics (Extant Respirometry)	C	As required	1 L polypropylene bottle	2-5	200	4°C	1
Biomass concentration Volatile suspended solids	C,G	8	1 L polypropylene bottle	1-5	1000	4°C	7

Sample Location (See Figure 3) : 1 - influent, 2 - primary effluent, 3 - final effluent, 4 and 4' - anoxic tank (trains 1 and 2), 5 and 5'- aerobic tank 3 (trains 1 and 2)
* : Conducted at the University of Connecticut
C : composite samples are flow weighted samples composed of grab samples obtained every 3 h. Four composite samples are taken every week.
G : Grab sample
Ψ : Storage at 4°C. However, the biomass is removed from the sample via centrifugation at 3500 g for 10 minutes. Biomass removal arrests further biochemical oxidation of NH_4^+-N and NO_2^--N.

Flow proportioned composite samples will consist of samples taken every three hours over a twenty four hour period by plant operators. The average dynamics of nitrogen removal will be based on analysis of the flow proportioned composite samples. Grab sampling will also be performed to determine time-varying dynamics of biological nitrogen removal. By periodic sampling and nitrification kinetics testing, the baseline kinetics will be determined. Trends and variability in kinetic parameter estimates for the Monod constants describing nitrification and denitrification will be ascertained and input to the simulation and modeling package BIOWIN®. The model performance output will be correlated to actual measured performance. In addition, we will conduct retrospective sampling, wherein samples taken during episodes of nitrogen removal failure will be subject to the bulk chemical parameter characterization and inhibition kinetics estimation. This sampling effort will assist in the validation of the bulk measurements, QSARs, and the kinetic assays.

Facilities. For conducting the periodic assays required in this study, facilities available in the Environmental Processes Laboratory at the University of Connecticut include :
Batch respirometric station for kinetics measurement, Biological dissolved oxygen Monitor (YSI model 5300), Clark type polarographic dissolved oxygen probes (YSI model 5331), glass jacketed respirometric vessels, constant temperature controller and circulator (Fisher Isotemp 9501), Hach COD Digester (COD Reactor Model 45600), high speed centrifuges (Marathon 22KBR and Sorvall RC-5C+), fixed wavelength spectrophotometer (Spectronic 20+ and 20D+), High performance liquid chromatograph station (Jasco Instruments), Ion chromatograph station (Dionex, Model A500), Perkin Elmer CHNSO analyzer (Series II, Model 2400), Ion specific electrodes for nitrate (Hach) and copper (Orion), gas sensing ammonia electrode (HNU systems).

At the Stamford WPCA, are available a COD (Hach) and nutrients analysis station (SKALAR) and a respirometric station identical to the one at the University of Connecticut. The personnel at Stamford have already been introduced to the extant respirometric technique for measuring nitrification kinetics via a workshop held at the University of Connecticut in August, 2000. Both respirometers at the University of Connecticut and Stamford will be used to monitor nitrification kinetics.

Related Research. The demand for nutrient removal, especially nitrogen, from wastewaters has been a demand placed on publicly and privately owned treatment works for over a decade. The general process engineering requirements to attain nitrogen removal via the concerted activities of nitrification and denitrification are well documented and implemented [7]. Nevertheless, even with apparent adequate process engineering in place, nitrogen removal is often unreliable[1]. Although some site-specific assessment of nitrification and/or denitrification kinetics limitations continue to be performed [22-24], design of such studies permit little generalization. On the other hand, several researchers have studied in great detail the kinetics and inhibition of nitrification in pure culture and clean matrices [9, 10, 25-34]. These studies, although of significant inherent scientific value, are of limited relevance to situations of interest that involve complex matrices and mixed microbial activities. Our kinetic assays [3, 4] are similar to, and compete with similar assays that have been designed by researchers overseas [35-41]; our assays to measure bulk inhibitory character of wastewaters are truly novel. Hence, we believe that our study, commenced with funding from the LISS program, is truly on the forefront for the development of predictive tools to assess nitrogen removal from wastewater in treatment facilities. Our study combines a strong scientific based method development with a full-scale real-world evaluation of our methodology.

- [1] **Chandran, K.**, and B. F. Smets. **2000**. "Factors Limiting Biological Nitrogen Removal : Nitrification Inhibition and Nitrogen Availability". *New England Water Environment Association (NEWEA) Technical Specialty Seminar on* **Biological Nutrient Removal in New England**. *Storrs, CT.*

Principal Results and Significance

Chemical analtyical MEASURES OF Nitrification Inhibitory character

We are developing a rapid assay to determine the total concentration of heavy metal cations in a wastewater. The heavy metal cations are precipitated as metal sulfides by titrating the wastewater sample with sodium sulfide. We quantify the total concentration of metal cations in the wastewater sample by measuring sulfide consumption during the assay.
We have calibrated the bulk metal assay to measure heavy metal concentrations using metal solutions in a deionized water matrix at pH 7.0. We are currently verifying the developed method in the feed medium to nitrifying bench-scale reactors. Subsequent verification will employ primary clarifier effluent at the Stamford WPCA as the test matrix. Following calibration and verification, we will apply test heavy metal solutions to both the bulk metal assay and the nitrification biokinetic assay and correlate the measured total heavy metal concentration to calculated heavy metal speciation and measured nitrification inhibition.

Operation of Bench-Scale BNR Reactors
Fabrication of the bench-scale reactors to be installed at the Stamford WPCA was performed by the University of Connecticut Technical Services Center and completed in March 2001. Bench-scale operation at Stamford is expected to commence during June 2001. Upon installation at the Stamford WPCA, the reactors will be seeded with biomass from the full-scale reactors at the same facility. Continuous monitoring of reactor performance and kinetics to infer biological nitrogen removal performance and BNR inhibition will commence after the reactors attain steady state performance.

Evaluation of Full-Scale BNR performance
Continuous monitoring of the full-scale reactors at Stamford commenced on November 6, 2001 and is being conducted in accordance with the schedule and particulars described in the monitoring proposal. Full-scale reactor monitoring will continue through November 2001 to enable collection of pertinent data across a wide range of seasonal, wastewater composition and biocatalyst activity dynamics.

Project Personnel Supported
Kartik Chandran Ph.D., Post Doctoral Fellow, Environmental Engineering Program, University of Connecticut.
Monika Siwek, Undergraduate student, Microbiology Program, University of Connecticut.
Wojciech Krach, Undergraduate student, Environmental Engineering Program, University of Connecticut.

Literature Cited

1. USEPA, *Long Island Sound Study. Phase III Actions for Hypoxia Management. EPA 902-98-R-002,* . 1998, EPA Long Island Sound Office: Stamford, CT; Stony Brook, NY.
2. Johnson, G., *A Sound Solution.* Water Environment and Technology, 1999(July): p. 47-51.
3. Chandran, K. and B.F. Smets, *Single Step Nitrification Models erroneously describe batch ammonia oxidation profiles when nitrite oxidation becomes rate limiting.* Biotechnology & Bioengineering, 2000. ***68***(4): p. 396-406.
4. Chandran, K. and B.F. Smets, *Applicability of two-step models in estimating nitrification kinetics from batch respirograms under different relative dynamics of ammonia and nitrite oxidation.* Biotechnology and Bioengineering, 2000. **70**(1): p. 54-64.
5. Sharma, B. and R.C. Ahlert, *Nitirification and Nitrogen Removal.* Water Research, 1977. **11**: p. 897-925.
6. Knowles, R., *Denitrification.* Microbiological Reviews, 1982. **46**(1): p. 43-70.
7. Grady, C.P.L.J., G.T. Daigger, and H.C. Lim, *Biological Wastewater Treatment.* 2 ed. Environmental Science and Pollution Control Series. 1999, New York: Marcel Dekker.
8. Blum, D.J.W. and R.E. Speece, *A Database of Chemical Toxicity to Environmental Bacteria and its use in Interspecies Comparisions and Correlations.* Research Journal of the Water Pollution Control Federation, 1991. **63**(3): p. 198-207.
9. Bedard, C. and R. Knowles, *Physiology, Biochemistry and Specific Inhibitors of CH_4, NH_4^+ and CO Oxidation by Methanotrophs and Nitrifiers.* Microbiological Reviews, 1989. **53**(1): p. 68-84.

10. Hooper, A.B. and K.R. Terry, *Specific Inhibitors of Ammonia Oxidation in Nitrosomonas.* Journal of Bacteriology, 1973. **115**(2): p. 480-485.

11. Hockenbury, M.R. and C.P.L.J. Grady, *Inhibition of Nitrification - Effects of Selected Organic Compounds.* Journal of the Water Pollution Control Federation, 1977: p. 768-777.

12. Chandran, K., *Biokinetic Characterization of Ammonia and Nitrite Oxidation by a Mixed Nitrifying Culture using Extant Respirometry. Ph. D. Thesis*, . 1999, University of Connecticut: Storrs.

13. Ellis, T.G., *et al.*, *Respirometric Technique for Determination of Extant Kinetic Parameters Describing Biodegradation.* Water Environment Research, 1996. **68**(5): p. 917-926.

14. Gee, C.S., M.T. Suidan, and J.T. Pfeffer, *Modeling of Nitrification under Substrate-Inhibiting Conditions.* Journal of Environmental Engineering, 1990. **116**(1): p. 18-31.

15. Mauret, M., *et al.*, *Application of Experimental Research Methodology to the Study of Nitrification in Mixed Culture.* Water Science and Technology, 1996. **34**(1-2): p. 245-252.

16. Hugo, W.B., *Disinfection Mechanisms*, in *Principles and Practice of Disinfection, Preservation and Sterilization*, A.D. Russel, W.B. Hugo, and G.A.J. Ayliffe, Editors. 1992, Blackwell Scientific Publications: Oxford.

17. Dean, J.A., ed. *Lange's Handbook of Chemistry*. 13 ed. . 1985, McGraw-Hill Book Company: New York, NY.

18. Hugo, W.B. and A.D. Russell, *Types of Antimicrobial Agents*, in *Principles and Practice of Disinfection, Preservation adn Sterilization*, A.D. Russell, W.B. Hugo, and G.A.J. Ayliffe, Editors. 1992, Blackwell Scientific Publications: Oxford.

19. Weiner, N.D., F. Hart, and G. Zografi, *Application of the Feruson Principle to the Antimicrobial Activity of Quarternary Ammonium Salts.* Journal of Pharmacy and Pharmacology, 1965. **17**: p. 350-355.

20. Nirmalakhandan, N., *et al.*, *Structure- and Property- Activity Relationship Models for Prediction of Microbial Toxicity of Organic Chemicals to Activated Sludge.* Ecotoxicology and Environmental Safety, 1998. **39**: p. 112-119.

21. Eaton, A.D., L.S. Clesceri, and A.E. Greenberg, eds. *Standard Methods for the Examination of Water and Wastewater*. 19 ed. . 1995, APHA, AWWA and WEF: Washington DC.

22. Fillos, J., *et al. Determination of Nitrifier Growth Rates at NYC Water Pollution Control Plants.* in *WEFTEC 2000*. 2000. Anaheim, CA.

23. Kaempfer, H., *et al. Calibration and Verification of a Biological Nutirent Removal for a High Strength Wastewater*. in *WEFTEC 1999*. 1999. New Orleans, LA.

24. Stensel, H.D. and G. Horne. *Evaluation of Denitrification Kinetics at Wastewater Treatment Facilities.* in *WEFTEC 2000*. 2000. Anaheim, CA.

25. Alleman, J.E. *Respiration-Based Evaluation of Nitrification Inhibition using Enriched Nitrosomonas Cultures.* in *International Conference on Innovative Biological Treatment of Toxic Wastewaters.* 1986. Arlington, VA.

26. Alleman, J.E., V. Keramida, and L.P. Kiser, *Light Induced Nitrosomonas Inhibition.* Water Research, 1987. **21**(4): p. 499-501.

27. Hyman, M.R., I.B. Murton, and D.J. Arp, *Interaction of Ammonia Monooxygenase from Nitrosomonas europaea with Alkanes, Alkenes and Alkynes.* Applied and Environmental Microbiology, 1988. **54**(12): p. 3187-3190.

28. Iizumi, T., M. Maizumoto, and K. Nakamura, *A Bioluminiscence Assay Using Nitrosomonas europaea for Rapid and Sensitive Detection of Nitrification Inhibitors.* Applied and Environmental Microbiology, 1998. **64**(10): p. 3656-3662.

29. Loveless, J.E. and H.A. Painter, *The Influence of Metal Ion Concentration and pH Value on the Growth of a Nitrosomonas Strain Isolated from Activated Sludge.* Journal of General Microbiology, 1968. **52**: p. 1-14.

30. Neufeld, R.D., A.J. Hill, and D.O. Adekoya, *Phenol and Free Ammonia Inhibtion to Nitrosomonas Activity.* Water Research, 1980. **14**: p. 1695-1703.

31. Stein, L.Y. and D.J. Arp, *Loss of Ammonia Monooxygenase Activity in Nitrosomonas europaea upon Exposure to Nitrite.* Applied and Envionmental Microbiology, 1998. **64**(10): p. 4098-4102.

32. Hyman, M.R., C.Y. Kim, and D.J. Arp, *Inhibition of Ammonia monooxygenase in Nitrosomonas europaea by Carbon Disulfide.* Journal of Bacteriology, 1990. **172**: p. 4775-4782.

33. Logan, M.S.P., C. Balny, and A.B. Hooper, *Reaction with Cyanide of Hydroxylamine Oxidoreductase of Nitrosomonas europaea.* Biochemistry, 1995. **34**: p. 9028-9037.

34. Rasche, M.W., M.R. Hyman, and D.J. Arp, *Factors Limiting Aliphatic Chlorocarbon Degradation of Nitrosomonas europaea: Cometabolic Inactivationof Ammonia Monooxygenase and Substrate Specificity.* Applied and Environmental Microbiology, 1991. **57**: p. 2986-2994.

35. Vanrolleghem, P.A., *et al., An On-Line Respirometric Biosensor for the Characterization of Load and Toxicity of Wastewaters.* Journal of Chemical Technology and Biotechnology, 1994. **59**: p. 321-333.

36. Kong, Z., P. Vanrolleghem, and W. Verstraete, *Automated Respiration Inhibition Kinetics Analysis (ARIKA) with a Respirographic Biosensor.* Water Science and Technology, 1994. **30**(4): p. 275-284.

37. Vanrolleghem, P.A. and F. Coen, *Optimal design of in-sensor experiments for on-line modeling of nitrogen removal processes.* Water Science and Technology, 1995. **31**(2): p. 149-160.

38. Kong, Z., P. Vanrolleghem, and W. Verstraete, *Simultaneous Determination of Inhibition Kinetics of Carbon Oxidation and Nitrification with a Respirometer.* Water Research, 1996. **30**(4): p. 825-836.

39. Surmacz-Gorska, J., *et al., Nitrification Monitoring in Activated Sludge by Oxygen Uptake Rate (OUR) Measurements.* Water Research, 1996. **30**(5): p. 1228-1236.

40. Gernaey, K., et al., A Titration Technique for Online Nitrification Monitoring in Activated Sludge. Water Science and Technology, 1998. **37**(12): p. 103-110.

41. Vanrolleghem, P.A. and W. Verstraete, Simultaneous Biokinetic Characterization of Heterotrophic and Nitrifying Populations of Activated Sludge with an On-line Respirographic Biosensor. Water Science and Technology, 1993. **28**(11-12): p. 177-187.